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VOLUME REGULATION AND NITROGEN METABOLISM IN THE MURICID GASTROPOD *THAIS HAEMASTOMA*

M. A. KAPPER[1,**], W. B. STICKLE[1], AND E. BLAKENEY[2,*]

[1]*Department of Zoology and Physiology,* [2]*Department of Biochemistry, Louisiana State University, Baton Rouge, Louisiana 70803*

Abstract

Ammonia and primary amine excretion and concentrations of intracellular ninhydrin-positive substance (NPS) and free amino acids (FAA) were measured in *Thais haemastoma* acclimated to salinities between 5 and 35‰ and over 14 days following direct transfer from 10 to 30‰ or from 30 to 10‰. There was no trend in excretion rates with acclimation salinity. Intracellular NPS and FAA levels were directly related to acclimation salinity, with amino acids constituting over 90% of the NPS at salinities greater than 10‰. The intracellular free amino acid pool of *T. haemastoma* was not dominated by any single amino acid but glycine, alanine, aspartate, taurine, proline, and glutamate (in decreasing order) each contributed more than 5% of the FAA. Alanine and glycine were the major intracellular osmotic effectors during both the high and low salinity transfers. Taurine levels did not change in the hyperosmotic transfer, but taurine was lost from the foot over the course of the hyposmotic transfer, suggesting that it behaves as a passive osmolyte. Snails are capable of taking up exogenous ammonia from seawater during a 10 to 30‰ transfer, suggesting that ammonia is being used as an aminating source.

Introduction

The southern oyster drill *Thais haemastoma* (Gray, 1839) is exposed in the field to both diurnal salinity fluctuations between 15 and 30‰ and extended periods of relatively constant salinity (Hewatt, 1951; Barrett, 1971). Even though its low salinity distributional limit in nature is 15‰, it will survive for over four weeks at salinities as low as 5–7.5‰ (Garton and Stickle, 1980; Hildreth and Stickle, 1980) and maintains a positive energy budget throughout the salinity range at temperatures greater than 15°C (Stickle, 1985a).

As is true of other marine molluscs, the hemolymph of *T. haemastoma* remains isosmotic to ambient seawater (Hildreth and Stickle, 1980; Stickle and Howey, 1975). The predominant labile intracellular osmolytes in marine molluscs are organic compounds. In many species studied to date, these are free amino acids (Burton, 1983), but changes in the intracellular free amino acid pool of several gastropods appear to be insufficient to account for the changes in intracellular osmolality following a change in ambient salinity (Schoffeniels and Gilles, 1972; Polites and Mangum, 1980). Inorganic ions and quaternary ammonium compounds such as glycine betaine and proline betaine have recently been identified as the primary labile intracellular osmolyte in some species (Pierce *et al.*, 1983).

The objectives of the present study were to (1) determine the degree of volume regulation and the changes in the patterns of nitrogen excretion in *T. haemastoma*

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* Current address: Department of Chemistry, Centenary College, Shreveport, Louisiana 71104.

** Current address: Zoology Department, Iowa State University, Ames, Iowa 50011.

during high and low salinity adaptation, (2) determine the changes in the free amino acid pool during adaptation to altered salinities, and (3) determine the extent to which changes in the free amino acid pool are responsible for salinity adaptation.

Materials and Methods

Collection and acclimation of animals

Snails were collected from pilings and bulkheads in the vicinity of Caminada Pass near Grand Isle, Louisiana, transferred to Baton Rouge, and placed into 38-liter aquaria containing artificial seawater (ASW; Instant Ocean, Mentor, Ohio) of the same temperature and salinity (30°C, 27‰) as in the field. Salinity adaptation was accomplished by adding either deionized water or concentrated ASW to change salinity by 2‰ per day. Animals were held at the final salinities for two weeks before being used. Small oysters (*Crassostrea virginica*) were provided as prey.

NPS and free amino acids

Ninhydrin-positive substances (NPS) and free amino acid levels were measured in the foot tissue of snails acclimated to 5, 7.5, 10, 15, 20, 25, 30, and 35‰ at 30°C, and directly transferred from 10 to 30‰ or from 30 to 10‰. Measurements were taken on transferred snails (n = 10) on days 0, 1, 2, 3, 7, 10, and 14 after transfer. NPS and free amino acids were also measured in the foot tissue of snails used in the ammonia-loading experiments described later.

Foot tissue was excised, then frozen in liquid nitrogen and lyophilized. After grinding in a Wiley mill, 10 mg of tissue were leached in 5 ml of 5-sulfosalicylic acid for 48 h. Samples were centrifuged at 20,000 × *g* for 15 min and the supernatant was assayed for NPS according to Rosen (1957). Concentrations of individual amino acids were determined on a Beckman Model 119 amino acid analyzer.

Ammonia and primary amine excretion and activity

The rate of ammonia and primary amine exchange was measured by incubating snails in 150 ml of ASW for 60 min and analyzing the incubation medium by the Solorzano (1969) phenol-hypochlorite method (ammonia) and North's (1975) fluorescamine technique (amines). Ammonia exchange is defined as the sum of NH_3 and NH_4^+ exchange. Since urea makes up an appreciable fraction of the excreta in some carnivorous marine invertebrates (Stickle, 1985b), further samples of the incubation medium were analyzed for urea by the Sigma urea assay (Sigma technical bulletin #640) at 10 and 30‰. All glassware used in excretion measurements had been baked at 450°C in a muffle furnace to eliminate exogenous amines. Excretion was measured for snails acclimated to each steady state salinity and at hours 3, 6, 9, and 12, and days 1, 2, 3, 4, 5, 6, 7, 10, and 14 after transfer from 10 to 30‰ or from 30 to 10‰.

Further experiments were designed to test the ability of *T. haemastoma* to take up exogenous ammonia from the medium for use as a possible aminating source during high salinity acclimation. The ammonia excretion rate of snails acclimated to 10 and 30‰ was measured using incubation water containing various concentrations of NH_4Cl up to 350 μM. These served as a control to see if snails would normally take up ammonia from ambient seawater. Then snails acclimated to 10‰ were placed in a chamber through which 30‰ water was pumped. The high salinity water flowing through the cell contained 0, 35, 45, 100, 175, or 350 μM NH_4Cl. Ammonia excretion

or uptake was measured after 24 h for the 35, 45, and 100 μM spiked animals, and in the time intervals of 8–12 h and 20–24 h after the transfer for animals subjected to either 0, 175, or 350 μM NH_4Cl. Foot tissue was sampled for NPS and FAA determination from 10‰ acclimated snails (controls) and at h 12 and 24 after transfer from snails in the 0, 175, and 350 μM NH_4Cl spiked transfers.

In both sets of salinity transfer experiments, a snail's activity was assigned a value of 1.0 if its foot was extended and attached to the substrate, 0.5 if the foot was extended but not attached, and 0 if the foot remained withdrawn.

Body water determination

The amount of water in the soft tissues at each salinity was determined as the difference in the weight of the soft parts before and after lyophilyzation. Oglesby's (1975) beta value was calculated at each steady state salinity as an indicator of the degree of regulation of body water content.

Statistical analyses

The General Linear Model procedure and Duncan's Multiple Range option of the Statistical Analysis System (SAS Institute, 1982) were used in data analysis. A probability level of 0.05 was significant.

Results

Steady state experiments

Levels of ninhydrin-positive substances in foot tissue of *Thais haemastoma* were directly related to the acclimation salinity over the range of 5–35‰ when expressed in terms of μmoles · g dry tissue weight^{-1} or μmoles · g tissue water^{-1} (Fig. 1).

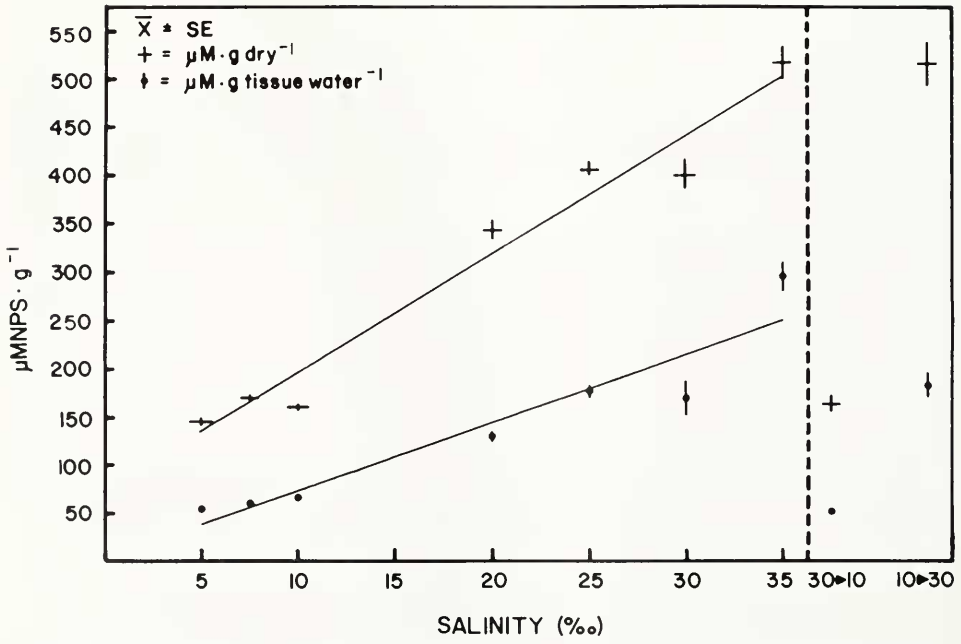

FIGURE 1. Ninhydrin-positive substance concentrations ($\bar{x} \pm$ S.E., n = 12) in the foot tissue of *Thais haemastoma* expressed as μmoles NPS · g dry tissue weight^{-1} (+) and as μmoles NPS · g body water^{-1} (●).

The free amino acid composition of the foot tissue of *T. haemastoma* at different acclimation salinities is given in Table I. Free amino acids comprise over 91% of the NPS pool at acclimation salinities of 10‰ and above. At 5 and 7.5‰ free amino acids account for 72 and 61% of the NPS, respectively. Other non-amino acid nitrogenous substances make up a significant portion of the NPS pool at very low salinities.

Excretion rates of snails acclimated to constant salinities varied among salinities (Fig. 2). Ammonia excretion rates were significantly greater than zero, but did not show a linear trend across salinities. The rate of ammonia excretion was higher at 15 and 20‰ than at any of the other acclimation salinities. The rate of primary amine exchange in snails acclimated to 5, 7.5, and 30‰ was in the uptake direction from the incubating medium. There was no significant exchange of amines between the animals and the medium at either 10 or 35‰, and the snails excreted amines at a constant rate of 0.17 μmole $\cdot$ g^{-1} $\cdot$ h^{-1} between 15 and 25‰. The rates of urea excretion at 10 and 30‰ were 0.17 $\pm$ 0.07 and 0.20 $\pm$ 0.06 μmoles $\cdot$ g dry weight^{-1} $\cdot$ h^{-1}, respectively, representing a minimal contribution to total nitrogen excretion.

Thais haemastoma is an excellent regulator of tissue water content. When acclimated to constant salinity between 5 and 30‰ the percentage of the fresh weight of the soft tissues consisting of water is not significantly different, and is only slightly lower at 35‰ (Table II).

Direct transfer experiments

Changes in percent body water over the 14 days of a 10 to 30‰ and a 30 to 10‰ transfer are shown in Figure 3. During the 10 to 30‰ transfer, body water declined from 79.3 $\pm$ 0.5% on day 0 to 72.7 $\pm$ 0.9% on day 2. By day 3 the percent body water had stabilized, and was not significantly different from the 30‰ control. For the 30 to 10‰ transfer, percent body water increased from 71.1 $\pm$ 3.8% on day 0 to 80.2 $\pm$ 1.3% on day 2, was not significantly different from the 10‰ control by day 3. The beta value was 0.13 two weeks following transfer to either high or low salinity. Unlike the animals used for steady-state determinations of body water content, the snails used in these experiments showed a significant (α = 0.05) difference in percent body water on day 0 (before being transferred) between 10 and 30‰ yielding a beta value of 0.18. This is still a very low value for beta and is indicative of excellent regulation of body water content.

Fourteen days after being directly transferred from 30 to 10‰, the concentration of foot NPS was not significantly different from the 10‰ steady state value of 161 $\pm$ 3 μmoles $\cdot$ g dry weight^{-1} (Fig. 1). Two weeks after transfer from 10 to 30‰ the concentration of NPS in foot tissue was significantly higher than the steady state value at 30‰, but not from the steady state value at 35‰ (Fig. 1).

With the exception of glutamate and taurine, the concentrations of each amino acid in foot tissue of snails directly transferred from 10 to 30‰ was higher 14 d after the transfer than in foot tissue of snails acclimated to the 30‰ steady state (Table III). The largest discrepancy between the concentrations of any amino acid between the steady state and post-transfer conditions occurred with alanine, whose concentration reached 237 μmoles $\cdot$ g dry weight^{-1} on day 3 after the transfer, as compared to only 55 μmoles $\cdot$ g dry weight^{-1} in animals acclimated to 30‰.

Arginine and aspartate were the only two amino acids whose concentration did not decline over the course of the 30 to 10‰ transfer (Table IV). For each of the other amino acids, the concentration two weeks after the hyposmotic transfer was similar to the concentration in animals maintained at 10‰, except for serine and threonine, which were not detected in the 10‰ acclimated animals, but were present in small amounts in the 30 to 10‰ transfers. Alanine, glycine, and glutamate all showed tran-

TABLE I

Concentrations of amino acids in foot tissue of Thais haemastoma *adapted to constant salinities* [*μmoles · dry weight*$^{-1}$, $\bar{x} \pm$ *(S.E.), n = 6, to nearest μmole*]

Amino acid	Salinity (‰)															
	5	%	7.5	%	10	%	15	%	20	%	25	%	30	%	35	%
P-Serine	9 ± 1	9	6 ± 1	7	6 ± 0	4	7 ± 1	2	9 ± 0	3	5 ± 0	1	6 ± 0	2	N.D.	—
Taurine	N.D.	—	3 ± 0	4	12 ± 0	8	26 ± 2	6	43 ± 2	14	42 ± 1	11	42 ± 2	11	24 ± 0	5
Aspartate	25 ± 1	24	16 ± 2	15	34 ± 3	22	48 ± 5	10	44 ± 2	14	47 ± 1	12	50 ± 2	14	32 ± 4	7
Threonine	N.D.	—	N.D.	—	N.D.	—	4 ± 0	1	5 ± 1	2	6 ± 1	2	5 ± 1	1	8 ± 2	2
Serine	N.D.	—	2 ± 0	2	3 ± 0	2	17 ± 3	4	8 ± 1	3	22 ± 2	6	15 ± 1	4	30 ± 8	7
Glutamate	3 ± 0	3	10 ± 1	10	14 ± 1	9	25 ± 1	5	26 ± 2	8	26 ± 2	7	30 ± 1	8	34 ± 7	8
Proline	N.D.	—	N.D.	—	N.D.	—	68 ± 6	15	6 ± 0	2	31 ± 3	8	32 ± 6	9	41 ± 5	9
Glycine	2 ± 0	2	2 ± 0	2	3 ± 0	2	92 ± 2	20	35 ± 4	11	92 ± 6	24	78 ± 7	21	62 ± 4	14
Alanine	6 ± 0	6	10 ± 1	9	9 ± 1	6	104 ± 1	22	31 ± 3	10	52 ± 3	13	55 ± 5	15	113 ± 9	26
Lysine	5 ± 1	5	5 ± 0	5	5 ± 1	4	4 ± 0	1	7 ± 1	2	6 ± 1	1	8 ± 1	2	6 ± 0	2
Arginine	33 ± 2	32	31 ± 2	25	43 ± 3	28	28 ± 1	6	46 ± 3	14	30 ± 2	8	29 ± 1	8	22 ± 2	5
Others	20	19	29	11	21	15	40	8	54	17	24	7	18	5	70	15
Total	105		105		153		463		314		385		369		441	

N.D. = Not detected.

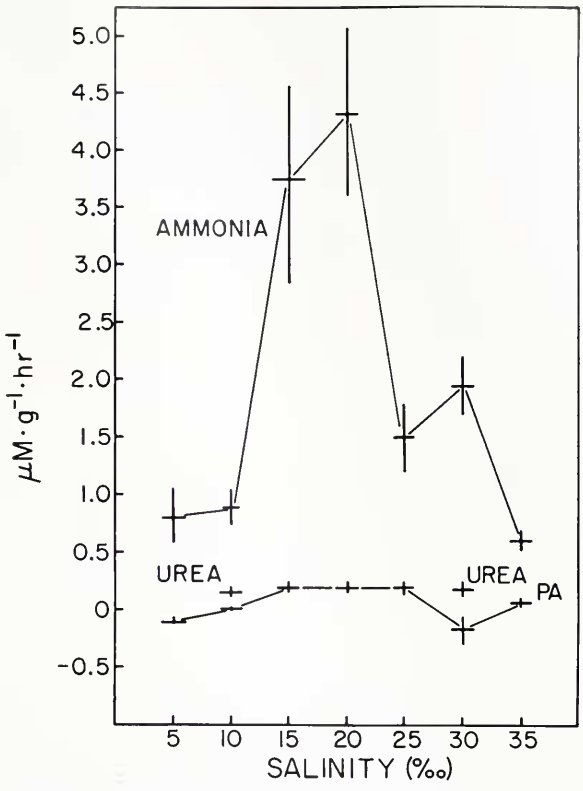

FIGURE 2. Nitrogen excretion rates (ammonia, primary amines and urea) in μmoles · g dry weight^{-1} · h^{-1} ($\bar{x} \pm$ S.E., n = 12) of *Thais haemastoma* acclimated to steady state salinities.

sient increases in concentration (μmoles · g dry weight^{-1}) over the first one to two days of the 30 to 10‰ transfer. This time corresponds to the time when the snails were withdrawn into their shells with the opercula closed (Fig. 4A).

Motor activity, and primary amine and ammonia excretion all dropped to nearly zero during the 12 hours immediately following a salinity transfer from 10 to 30‰

TABLE II

Percent tissue water in Thais haemastoma *as a function of acclimation salinity.* [$\bar{x} \pm$ *S.E. (n)*]

Sal	% Body water	DMR
5	72.9 ± 1.88 (12)	A
7.5	74.2 ± 0.74 (12)	A
10	70.98 ± 0.47 (12)	A
15	67.43 ± 0.57 (12)	A
20	72.34 ± 0.50 (30)	A
25	69.77 ± 0.85 (12)	A
30	72.01 ± 0.51 (24)	A
35	63.51 ± 0.84 (11)	B

Percent tissue water is not significantly different for those salinities sharing a common letter according to Duncan's Multiple Range Test (DMR).

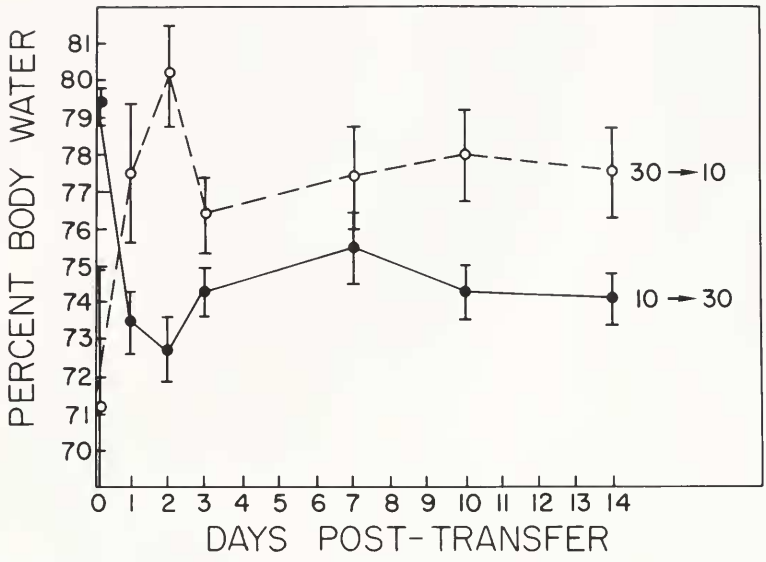

FIGURE 3. Changes in the percent body water ($\bar{x} \pm$ S.E., n = 6) of *Thais haemastoma* over time after direct transfer from 30 to 10‰ (○), and from 10 to 30‰ (●).

(Fig. 4) or from 30 to 10‰ (Fig. 5). After transfer from 10 to 30‰, activity of the snails remained low over the first 24 h. By day 3 all of the snails had reattached to the substrate indicating a normal activity pattern (Fig. 4A). Primary amine excretion fell during the 12 hours after the 10 to 30‰ transfer and slowly rose over the next 6 days. Amine loss fell to zero on day 10, and was not significantly different from control on day 14 (Fig. 4B). Ammonia excretion dropped precipitously immediately following the transfer and remained low for three days. Ammonia loss increased on days 4 and 5, and had returned to the control level by day 6 (Fig. 4C).

Snails remained unattached and withdrawn for the first 24 h after the 30–10‰ transfer (Fig. 5A). All had reattached by day 3 indicating a normal activity pattern (Fig. 5A). Amine excretion peaked on day 2 after transfer and returned to the control level by day 3 where it remained for the rest of the experiment (Fig. 5B). Ammonia excretion peaked on days 2–3 after transfer and remained fairly constant over days 4–7 before rising again by day 14 (Fig. 5C).

Ammonia loading

In snails acclimated to either 10 or 30‰, ammonia exchange with the medium was always in the direction of ammonia release, regardless of the amount of exogenous ammonia present. Twenty-four hours after snails were transferred from 10 to 30‰ there was a linear dose-related uptake of ammonia at exogenous ammonia concentrations up to 100 μM. At concentrations greater than 100 μM ammonia there was no change in the uptake rate (Fig. 6).

Over the first 24 hours of direct transfer from 10 to 30‰ the only amino acid to show any change in concentration was alanine (Table V), which, without exogenous ammonia in the high-salinity water, rose from 10 to 87 μmoles · g dry weight^{-1}. When either 175 or 350 μM ammonia was added to the high-salinity water, alanine levels rose at the same rate as in the control transfers for the first 12 h, but had leveled off

TABLE III

Free amino acid levels in foot tissue of Thais haemastoma *directly transferred from 10 to 30‰* [*μmoles · g dry weight*$^{-1}$ $\bar{x}$ (±S.E.) $n = 6$, *to nearest μmole*]

Amino acid	Day 0	Day 1	%	Day 2	%	Day 3	%	Day 7	%	Day 10	%	Day 14	%
P-Serine	12 ± 2	13 ± 3	8	8 ± 0	−33	9 ± 1	−25	8 ± 1	−33	12 ± 1	0	9 ± 1	−25
Taurine	33 ± 3	32 ± 1	−3	30 ± 2	−9	32 ± 1	−3	33 ± 3	0	40 ± 4	21	28 ± 2	−15
Aspartate	35 ± 3	12 ± 3	−66	49 ± 3	40	49 ± 4	40	49 ± 2	40	59 ± 1	69	59 ± 2	69
Threonine	4 ± 1	4 ± 0	0	23 ± 3	475	25 ± 3	525	35 ± 4	775	26 ± 3	550	31 ± 4	675
Serine	5 ± 1	5 ± 1	0	26 ± 2	420	31 ± 3	520	39 ± 4	680	38 ± 4	660	43 ± 4	760
Glutamate	11 ± 1	16 ± 3	45	23 ± 0	109	21 ± 1	91	25 ± 2	127	26 ± 2	136	19 ± 0	73
Proline	N.D.	4 ± 0	—	18 ± 3	350[a]	28 ± 4	600[a]	34 ± 5	750[a]	38 ± 4	850[a]	40 ± 2	900[a]
Glycine	3 ± 1	7 ± 0	133	28 ± 2	833	47 ± 5	1463	70 ± 5	2233	72 ± 6	2300	86 ± 2	1627
Alanine	15 ± 1	68 ± 5	353	181 ± 13	1107	237 ± 19	1488	220 ± 27	1367	177 ± 6	1080	202 ± 12	1247
Arginine	37 ± 3	45 ± 3	22	45 ± 2	22	38 ± 1	3	30 ± 2	−19	33 ± 2	−11	36 ± 3	−3
Others	14	44	214	37	164	38	171	33	136	41	193	47	236
Total	169	249	48	468	177	555	228	576	241	562	233	600	255

[a] Based on Day 1.
% = % change.
N.D. = Not detected.

TABLE IV

Free amino acid levels in foot tissue of Thais haemastoma *directly transferred from 30 to 10‰* [*μmoles · g dry weight*$^{-1}$ $\bar{x} \pm$ *(S.E.) n = 6, to nearest μmole*]

Amino acid	Day 0	Day 1	%	Day 2	%	Day 3	%	Day 7	%	Day 10	%	Day 14	%
P-Serine	6 ± 0	7 ± 1	17	8 ± 0	33	8 ± 1	33	10 ± 1	67	11 ± 1	83	12 ± 1	100
Taurine	82 ± 7	85 ± 3	4	88 ± 8	7	62 ± 2	−24	42 ± 2	−49	44 ± 3	−46	39 ± 2	−52
Aspartate	47 ± 4	41 ± 2	−13	50 ± 2	6	50 ± 3	6	36 ± 3	−23	41 ± 2	−12	39 ± 2	−17
Threonine	12 ± 2	13 ± 1	8	13 ± 2	8	8 ± 0	−33	3 ± 0	−75	3 ± 0	−75	2 ± 0	−83
Serine	20 ± 3	19 ± 1	5	24 ± 5	20	18 ± 3	−10	6 ± 0	−70	7 ± 1	−65	7 ± 1	−65
Glutamate	14 ± 1	20 ± 0	42	23 ± 1	64	23 ± 1	64	12 ± 1	−14	10 ± 2	−28	13 ± 1	−7
Proline	13 ± 2	21 ± 3	62	13 ± 3	0	5 ± 0	−61	N.D.	−100	N.D.	−100	N.D.	−100
Glycine	53 ± 8	72 ± 4	36	33 ± 9	−38	9 ± 2	−83	4 ± 0	−92	3 ± 0	−94	3 ± 0	−94
Alanine	42 ± 4	86 ± 6	105	38 ± 9	−10	24 ± 4	−43	10 ± 1	−76	9 ± 1	−79	12 ± 2	−71
Arginine	25 ± 2	28 ± 1	12	31 ± 1	24	32 ± 2	28	25 ± 2	0	36 ± 1	44	34 ± 2	36
Others	36	44	22	22	−39	31	−14	45	25	51	42	40	11
Total	350	436	25	343	−2	270	−23	193	−45	215	−39	201	−42

% = % change.
N.D. = Not detected.

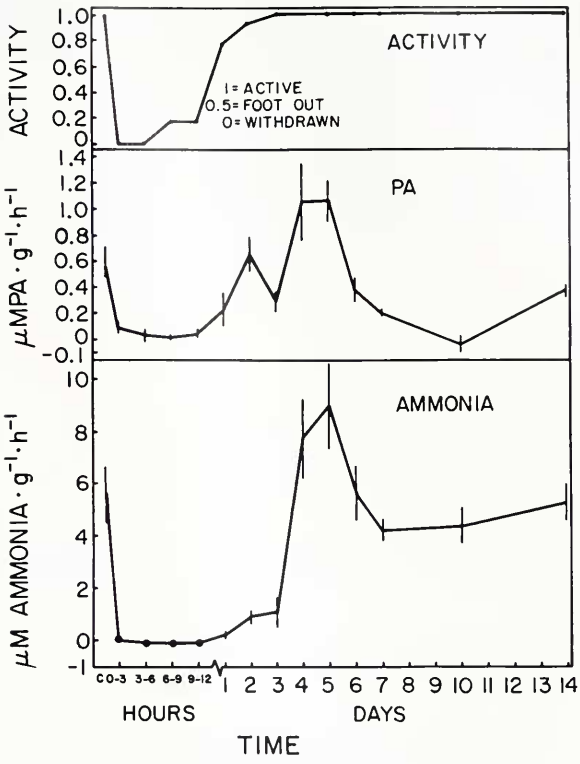

FIGURE 4. Activity (A, 0 = operculum closed, 0.5 = foot out, but not attached, 1.0 = foot out and attached to substrate), ammonia excretion (B, in μmoles · g dry weight^{-1} · h^{-1}) and primary amine excretion (C, in μmoles · g dry weight^{-1} · h^{-1}) over 14 days in *Thais haemastoma* directly transferred from 10 to 30‰. ($\bar{x} \pm$ S.E., n = 12).

by hour 24. The total FAA pool at 24 h after transfer is not significantly different in the NH_4^+-spiked transfers and in the control transfer due mainly to the very large variation in alanine concentration at 24 h in the control transfer (86.5 ± 15.8 μmoles · g dry weight^{-1}—Table V).

DISCUSSION

Thais haemastoma is a euryhaline species that partially regulates its volume by changes in the intracellular free amino acid pool. The FAA pool of *T. haemastoma* is composed of a mixture of seven quantitatively important free amino acids (Table I) rather than being dominated by a single free amino acid, such as taurine, as occurs in *T. lapillus* (Stickle *et al.*, in press). When subjected to altered salinity, volume regulation is achieved by the rapid initial alteration of the intracellular concentrations of alanine and glycine.

The water content of *Thais haemastoma* remained remarkably constant across acclimation salinities (Table II), and had returned to the steady state level two weeks after a direct salinity transfer in either direction between 30 and 10‰ (Fig. 3). Since the analysis of variance for water content *versus* salinity was not significant over the range of 5 to 30‰, we must assign a value of zero to Oglesby's (1975) beta. Hildreth

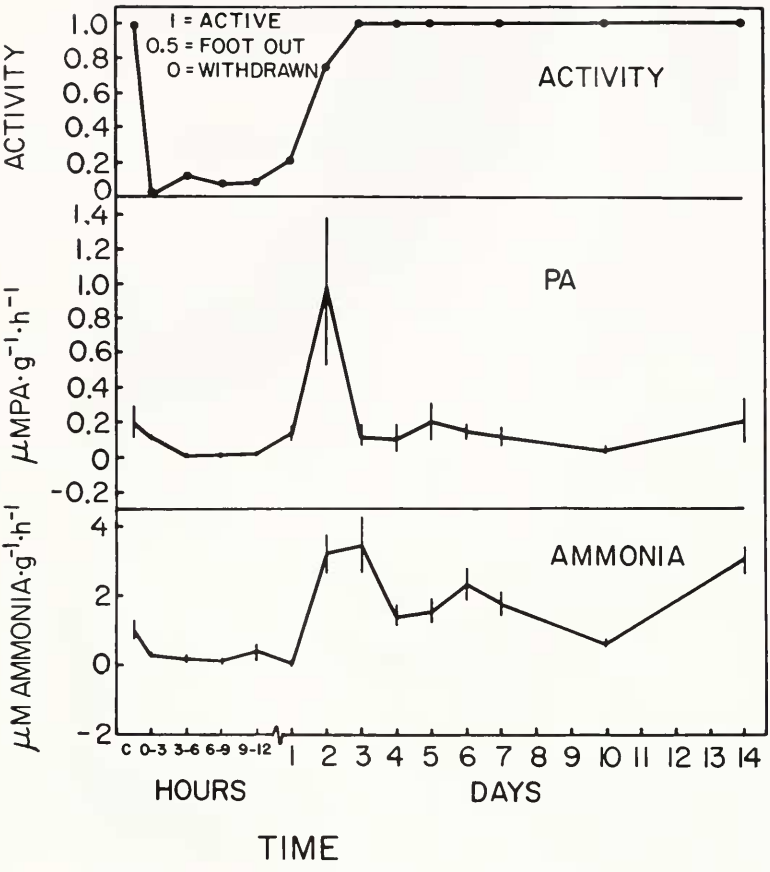

FIGURE 5. Activity (A, 0 = operculum closed, 0.5 = foot out, but not attached, 1.0 = foot out and attached to substrate), ammonia excretion (B, in μmoles · g dry weight^{-1} · h^{-1}) and primary amine excretion (C, in μmoles · g dry weight^{-1} · h^{-1}) over 14 days in *Thais haemastoma* directly transferred from 30 to 10‰. ($\bar{x} \pm$ S.E., n = 12).

and Stickle (1980) found a very small, yet statistically significant increase in body water content in *T. haemastoma* as the acclimation salinity was decreased from 30 to 10‰ from 72% to 77%, 10‰ yielding a beta of 0.07, still indicative of excellent volume regulation. This is in direct contrast to the pattern of body water regulation in *Thais lapillus,* where beta values range from 0.22 to 1.26, depending on the temperature (Stickle *et al.,* in press).

Although there is no significant change in the total amount of body water in snails acclimated to salinities between 5 and 30‰ and the hemolymph of *T. haemastoma* is isosmotic to ambient seawater (Stickle and Howey, 1975; Hildreth and Stickle, 1980), it is possible that the distribution of water between the intra- and extracellular compartments might show a reciprocal change with salinity. Staaland (1970) was able to measure the volume of the extracellular space (as inulin space) in *Buccinum undatum* acclimated to several salinities and found that as salinity was raised from 10 to 35‰, the size of the intracellular fluid compartment decreased as the size of the extracellular compartment increased. The total amount of body water decreased and the concen-

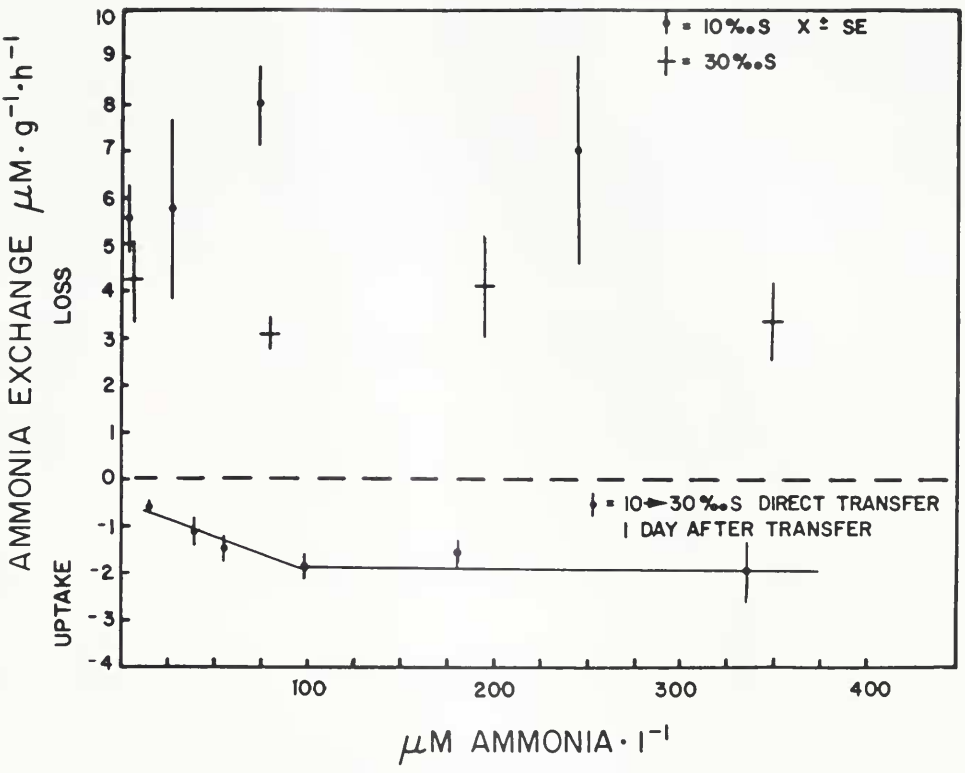

FIGURE 6. Ammonia excretion rates of *Thais haemastoma* (μmoles · g dry weight^{-1} · h^{-1}, $\bar{x} \pm$ S.E., n = 12) as a function of exogenous ammonia in the medium.

tration of intracellular NPS increased with the increasing salinities. Since the total body water in *T. haemastoma* remains constant between 5 and 30‰, it might be argued that changes in the FAA pool are only reflections of a constant amount of FAA being diluted or concentrated by varying amounts of intracellular water, and this would appear to be the case when FAA levels are expressed in terms of cellular hydration. Although we did not use an ECF marker in this study, it is reasonable to assume that as the acclimation salinity decreased the size of the intracellular compartment increased at the expense of the extracellular compartment. Indirect evidence for such a change is given by Findley *et al.* (1978) who report increasing difficulty in obtaining hemolymph samples from *T. haemastoma* as the exposure salinity decreased. Expressed on a dry weight basis, the increase in cellular FAA in *T. haemastoma* is real, indicating that these solute molecules are being used as osmotic effectors.

The percent body water in the snails used in steady-state salinity experiments was higher at both 10 and 30‰ than in those snails used in the direct transfer experiments, nearly a 10% difference at 10‰. The steady-state salinity experiments were done using snails collected in mid spring, and the direct transfer experiments were done using snails collected in mid-summer, before and after the breeding and capsule deposition season for this population of southern oyster drills (pers. obs.). Stickle (1973) found a seasonal variation in the body water index (g body water · g live weight^{-1} · 100) in a population of *T. lamellosa* acclimated to 30‰ from near Friday Harbor, Washington.

TABLE V

Free amino acid levels in the foot of Thais haemastoma *after direct transfer from 10 to 30‰ under conditions of ammonia loading*
[*μmoles · g dry weight*$^{-1}$ $\bar{x} \pm$ *(S.E.) n = 6, to nearest μmole*]

Amino acid	10‰	+0 μM				+175 μM				+350 μm			
		12 h	%	24 h	%	12 h	%	24 h	%	12 h	%	24 h	%
P-Serine	14 ± 0	13 ± 1	−7	12 ± 1	−14	13 ± 0	−7	15 ± 1	7	16 ± 2	14	14 ± 0	0
Taurine	24 ± 1	23 ± 2	−4	30 ± 3	25	23 ± 1	−4	25 ± 1	4	24 ± 0	0	28 ± 1	17
Aspartate	38 ± 1	6 ± 1	−84	32 ± 4	−16	10 ± 1	−74	5 ± 1	−87	5 ± 0	−87	17 ± 4	−55
Threonine	2 ± 0	2 ± 0	0	5 ± 0	160	1 ± 0	−50	1 ± 0	−50	2 ± 0	0	2 ± 0	0
Serine	5 ± 0	3 ± 0	−40	10 ± 2	100	3 ± 0	−40	3 ± 0	−40	3 ± 0	−40	3 ± 0	−40
Glutamate	11 ± 0	14 ± 0	27	18 ± 0	64	15 ± 1	36	9 ± 1	−18	12 ± 1	9	14 ± 0	27
Proline	N.D. —	4 ± 1	—	7 ± 0	75[a]	N.D. —	—	N.D. —	—	N.D. —	—	4 ± 1	0[a]
Glycine	4 ± 1	4 ± 0	0	7 ± 1	75	4 ± 1	0	3 ± 0	−25	3 ± 0	−25	6 ± 1	50
Alanine	10 ± 1	43 ± 5	330	86 ± 16	760	40 ± 3	300	48 ± 6	380	38 ± 3	280	51 ± 5	410
Arginine	47 ± 2	41 ± 2	−13	48 ± 1	2	50 ± 2	6	37 ± 4	−21	35 ± 1	−25	42 ± 1	−11
Others	8	18	11	25	212	14	75	13	62	13	62	20	150
Total	163	171	5	280	72	173	6	159	−2	151	−7	201	23

[a] Based on 0 μM 12 h.
% = % change.
N.D. = Not detected.

Giese (1969) found an inverse relationship between body water and lipid content in pre-spawning black abalone, *Haliotis cracheroidii,* and Belisle and Stickle (1978) suggest that seasonal changes in lipid concentration may mask a relationship between percent body water and salinity in *T. haemastoma.*

The free amino acid pool of *T. haemastoma* acclimated to steady state salinities is not dominated by any one amino acid; rather a combination of alanine, glycine, glutamate, aspartate, arginine, proline, and taurine combine to constitute up to 92% of the intracellular free amino acid pool. Of these, alanine and glycine are the most common, comprising up to 25% each of the FAA pool at 15‰. Alanine predominates in the FAA pool during high-salinity acclimation.

A single amino acid is often dominant in the FAA pool of marine invertebrates. Taurine dominates the FAA pool of many stenohaline gastropod species, making up approximately 75% of the FAA pool in a population of *Thais lapillus* from England (Stickle *et al.,* in press), and 52% in a population from France (Hoyeaux *et al.,* 1976). Similarly, taurine accounted for 38% of the FAA in *Littorina littorea,* 75% in *Patella vulgata* (Hoyeaux *et al.,* 1976), and 78% in *Thais emarginata* (Emerson, 1969). Glycine or alanine are often predominant in the intracellular FAA pool of euryhaline species, making up to 53% of the FAA in *Mytilus edulis,* 33% in *Scrobicularia plana* (Hoyeaux *et al.,* 1976), 33–75% in *Rangia cuneata* (Fyhn, 1976; Henry *et al.,* 1980), 45% in *Crangon crangon* (Weber and van Marrewijk, 1972), and 25% in *Thais haemastoma* (current study).

Taurine metabolism is poorly understood, but it appears that it is very slowly formed and catabolized in molluscs (Bishop *et al.,* 1983). Taurine is certainly being used as an osmotic effector in *T. haemastoma,* comprising 14% of the FAA pool at 20‰ (Table I). Taurine is not being formed in the cells during the first two weeks of the 10 to 30‰ transfer, its concentration actually decreasing slightly (Table III). Over the course of the 30 to 10‰ transfer, taurine behaves like the other labile amino acids, slowly leaving the cells with time after the salinity decrease (Table IV). Taurine comprises 75% of the FAA pool in *T. lapillus,* and its concentration remains constant at 300 μmoles · g dry weight^{-1} over the range of acclimation salinities between 17.5 and 35‰ (Stickle *et al.,* in press). It has been hypothesized that the low salinity tolerance of *T. lapillus* is due to the fact that taurine makes up such a large percentage of the FAA, leaving no sizable pool of mobile FAA available for adjustment of the intracellular osmotic pressure (Stickle *et al.,* in press).

In isolated ventricles of the ribbed mussel, *Modiolus demissus,* transferred from 12 to 36‰, osmoregulation was virtually complete within five days after the salinity transfer (Baginski and Pierce, 1975). The alanine concentration rose immediately after transfer and started to decline after eight days by which time glycine levels had started to rise. The taurine concentration, which had been similar to that of both alanine and glycine at 16‰ showed a very slow rise over the course of the 101 day experiment until its concentration was once again equal to alanine and glycine; each amino acid contributing about one third of the total free amino acid pool (Baginski and Pierce, 1975). Free alanine and glycine are clearly being used as immediate osmotic effectors, taurine apparently only contributing more as an osmotic effector during very long-term osmotic adaptation to high salinity.

Although we only followed our transfer experiments for 14 days, an immediate increase in the concentrations of alanine and glycine similar to that found in *M. demissus* (Baginski and Pierce, 1975) was seen during high-salinity adaptation in *T. haemastoma.* By the second day after the 10 to 30‰ transfer the concentrations of these two amino acids exceeded their concentrations in animals acclimated to 30‰ (Tables I, III). The tendency for alanine concentration to slowly decline after day 3

of the hyperosmotic transfer (Table III) suggests that true return of the FAA pool to steady state conditions in *T. haemastoma* takes much longer than two weeks following a transfer to higher salinity.

Alanine and glycine were also the most labile members of the free amino acid pool even in the hyposmotic transfer (Table IV). Concentrations of both alanine and glycine rose over the first 24 h of the 30 to 10‰ transfer; the opposite of the expected response. This increase in glycine and alanine levels took place during the time when the operculum was tightly closed in all individuals, with consequent isolation from the environment and probable anaerobic metabolism. The transient increase in intracellular glycine and alanine (Table III) over the first two days of the 30 to 10‰ transfer might be attributable to anaerobic metabolism. Alanine has been found to accumulate in *T. haemastoma* after 24 h in nitrogen-saturated water (Ellington, pers. comm.). When subjected to a diurnal salinity fluctuation cycle of either 10–30–10 or 30–10–30‰ the oxygen consumption rate of *T. haemastoma* dropped off markedly and the siphon was retracted during the course of the salinity change (Findley *et al.*, 1978).

The only essential free amino acid found in appreciable quantities in foot tissue of *Thais haemastoma* was arginine. Its concentration remained remarkably constant over the entire range of acclimation salinities tested (Table I). Even after a direct transfer from high to low or from low to high salinity the arginine concentration did not change (Tables III, IV). Somero and Bowlus (1983) noted that at physiological pH's, the guanidino group of arginine carries a positive charge, leading to a disruption of enzyme function at elevated levels of free arginine. It seems reasonable then that free arginine concentration be strictly controlled, especially since the phosphogen in molluscs is arginine phosphate. Somero and Bowlus (1983) suggested that one way arginine levels are controlled in molluscan tissue during periods of high metabolic demand is the formation of octopine from pyruvate and arginine. Octopine does not affect enzyme structure or function at physiological levels. Livingstone *et al.* (1983) have found significant levels of octopine dehydrogenase in *Thais (=Nucella) lapillus, Buccinum undatum,* and *Neptunea antiqua.* We have only found trace levels of octopine dehydrogenase activity in foot tissue of *Thais haemastoma* (Kapper and Stickle, unpubl.). If changes in free arginine concentrations are disruptive to metabolism in *T. haemastoma,* arginine concentration is regulated in some other way.

After a decrease in ambient salinity, cell volume is initially increased by the osmotic influx of water. Volume is restored by the expulsion of solute from the cells along with osmotically obligated water (Pierce and Amende, 1981). The fate of amines lost from the FAA pool during low salinity adaptation could be excretion, deamination, or transamination followed by excretion (Bishop, 1976). If amines are lost from the cells during low salinity acclimation, then one would expect to see at least a transient increase in the level of free amino acids in the hemolymph. Stickle and Howey (1975) found the hemolymph NPS of *T. haemastoma* to increase during the low salinity phase of a 24-hour 30–10–30‰ salinity fluctuation. Similarly, Livingstone *et al.* (1979), noted an slight increase in hemolymph amino acids and ammonia in *Mytilus edulis* when transferred from 30 to 15‰, as did Strange and Crowe (1979) *in Modiolus demissus.*

If free amino acids are released from the cells and deaminated during low salinity adaptation and the resulting amino group excreted, there would be a pulse of ammonia excretion soon after hyposmotic transfer. Lange (1964) termed this efflux of free amino acids or ammonia the regulatory step for volume regulation. There was a peak in ammonia and primary amine excretion on days two and three after transfer from 30 to 10‰ in *T. haemastoma* (Fig. 5).

There was also a peak in ammonia and free amino acid excretion during the second and third days of the 10 to 30‰ transfer (Fig. 4). The periods of increased nitrogen excretion after transfer occurred after the snails had begun to reopen their opercula and expose the foot to the external environment. It can be hypothesized that the animals are anaerobic during the time that their opercula are closed, and that nitrogenous metabolites are accumulating in the tissues. If the total amount of nitrogen excreted during the time the snails are isolated is compared to the total amount of excreta expected had closure not occurred, there is a deficit of nearly 380 μmoles $\cdot$ g^{-1} over the three day period. During the second three days of the experiment, while excretory rates were elevated, the total amount of nitrogen excreted was 95 μmoles $\cdot$ g^{-1} greater than would be expected had closure not occurred. This does not account for all of the deficit of the first two days, but if the snails are using primarily anaerobic pathways during this time, then it is reasonable to assume that the overall magnitude of metabolism and thus production of ammonia and primary amines is reduced (Gade, 1983). The excretory pulse after reopening then probably does represent a flushing of accumulated metabolites from the body.

The data regarding the use of exogenous ammonia as an aminating source during high-salinity adaptation are not conclusive. Snails acclimated to steady state salinities do not take up ammonia from the ambient water even at concentrations up to 350 μM. When exogenous ammonia is available during the course of high salinity acclimation, it is taken into the animal, apparently in some saturable fashion (Fig. 6). The fate of the ammonia taken up by the animal cannot be determined without ^{15}-N tracer experiments.

To summarize, *Thais haemastoma* partially adapts to increased or decreased salinities by changing the size of the intracellular free amino acid pool. During an increase in ambient salinity, increases in alanine and glycine concentrations account for much of the increased intracellular osmolality. Volume regulation, as evidenced by changes in the amount of total body water is mostly complete within three days of the transfer, as are the largest changes in cellular free amino acid concentrations. Longer than 14 days are required for the intracellular free amino acid profile to return to the steady state pattern after hyperosmotic transfer. After a decrease in ambient salinity, alanine, glycine, and taurine are lost from the cells, and volume regulation is complete by three days after the transfer. It is clear that changes in the concentrations of intracellular free amino acids contribute significantly to the salinity adaptation process in *Thais haemastoma,* but these changes are probably not the only mechanism used by this species to cope with altered salinity.

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